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ABSTRACT

Objectives: Biological predisposition for specific metastatic organs might differ between molecular subgroups of lung cancer. We aimed to assess the association between molecular status and metastatic organs at diagnosis in a nationwide stage IV non-squamous non-small cell lung cancer ((ns)-NSCLC) cohort.

Methods: All ns-NSCLC from 2013 that were stage IV at diagnosis were identified from the Netherlands Cancer Registry, which records information on metastatic organs at diagnosis. Tumors were matched to the Dutch Pathology Registry (PALGA) from which data on molecular status established in routine practice was extracted. Four molecular subgroups (EGFR+, KRAS+, ALK+, triple-negative) were identified. For each metastatic organ, proportions of tumors metastasized to this organ were, per molecular subgroup, compared to triple-negative tumors by multivariable logistic regression analyses (adjusted odds ratios (OR) with 95% confidence intervals (CI)), taking clinicopathological variables into account.

Results: 160 EGFR+ (exon 19 del, exon 21 L858R), 784 KRAS+, 42 ALK+, and 1008 triple-negative tumors were identified. Most frequent metastatic organs were the bone (34%), pleura (24%), lung (23%), and brain (22%). Compared to triple-negatives, EGFR+ tumors had more often metastases to the bone (31.5 vs 53.8%; OR 2.55 (95% CI 1.80–3.62)) and pleura (24.1 vs 37.5%; OR 2.06 (1.42–2.98)), and less often to the brain (22.0 vs 12.5%; OR 0.53 (0.32–0.88)) and adrenal glands (19.1 vs 7.5%; OR 0.46 (0.28–0.75)). Compared to triple-negatives, KRAS+ and ALK+ tumors had at diagnosis metastasized more often to the lung (20.3 vs 26.7%; OR 1.40 (1.12–1.76)) and the liver (13.1 vs 23.8%; OR 2.07 (1.00–4.32)), respectively.

Conclusion: NSCLC molecular status was associated with metastatic pattern at diagnosis. 54% of stage IV EGFR+ ns-NSCLC patients had bone metastases at diagnosis. These observational results are hypothesis generating, and call for a prospective study where EGFR+ patients are screened for bone metastases, and treated to prevent skeletal related events.

1. Introduction

Non-squamous non-small cell lung cancer ((ns)-NSCLC) is often driven by molecular alterations, such as Kirsten rat sarcoma mutations (KRAS+), epidermal growth factor receptor mutations (EGFR+), and anaplastic lymphoma kinase rearrangements (ALK+) in 25–30%, 10–15% and 5% of Caucasian ns-NSCLC patients, respectively [1,2]. EGFR+ is a favorable prognostic factor and predictive for response to EGFR tyrosine kinase inhibitors (TKI) [3]; ALK+ is predictive for response to ALK-TKI [4]. Currently no effective KRAS-targeted therapy is available.

The biological predisposition for specific metastatic organs might

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differ between molecular subgroups. Understanding these differences might have implications for adequate metastasis screening and (prophylactic) treatment decisions. The biological predisposition is best studied in treatment naïve stage IV patients to exclude bias from treatment. Patients with EGFR+ NSCLC, for example, have a longer overall survival (OS) compared to patients with EGFR-wildtype (wt) disease, resulting in a longer time span for metastases to develop. Several studies have suggested that the cumulative incidence of brain as well as bone metastases may be higher in patients with EGFR+ NSCLC as compared to EGFR-wt [5–7]. Studies (N = 189-1063) evaluating bone and brain metastases at initial stage IV diagnosis have reported conflicting results [8–11].

Due to the relatively low number of included patients and the single or dual-centre setting, the studies published thus far were unable to adequately address the question whether biological predisposition for metastatic organs differs between molecular subgroups [5,7–11]. Some studies investigated the cumulative incidence of metastases to specific organs, but not the burden of metastases at diagnosis [5–7]. Furthermore, most studies focused on one or two metastatic organs and/or molecular alterations (mostly EGFR+) [5,8,9,11].

To evaluate the association of molecular status with organs of metastases at diagnosis, we conducted a retrospective population-based study in the Netherlands by linking data of stage IV ns-NSCLC from the Netherlands Cancer Registry (NCR) to pathology data (including molecular analysis data established in routine practice) retrieved from the Dutch Pathology Registry (PALGA).

2. Patients and methods

2.1. Patient selection

In the Netherlands, all patients diagnosed with cancer are registered in the NCR, managed by the Netherlands Comprehensive Cancer Organisation (IKNL). Data in the NCR is actively collected from patient records according to standardized formats by trained data managers [12], and include, but is not limited to: gender, age at diagnosis, morphology code (ICD-O 3rd edition), TNM-stage (7th edition, 2013), diagnosis of previous malignancy, and organs of metastases at diagnosis. Organs of metastases are recorded according to documented clinical data (cTNM) with a maximum of three separate locations. In cases with ≥3 organs, two are recorded and the third is coded as ≥3. Organ count is irrespective of the number of metastases within this organ. Furthermore, the NCR has a non-mandatory variable for indicating whether ^{18}FDG-PET was used for staging. The Dutch NSCLC guideline [13] states that staging should involve a contrast-enhanced computed tomography (CT) of the chest and the upper abdomen including the adrenals. ^{18}FDG-PET is recommended for all patients eligible for therapy with curative intent. In daily practice, ^{18}FDG-PET and CT of the chest and upper abdomen are often combined in one session. Specific data per hospital were not available.

All stage IV adenocarcinomas and NSCLC not otherwise specified (NOS) diagnosed between January 1 2013 and December 31 2013 recorded in the NCR were selected. To exclude possible bias due to previous treatment, only patients with stage IV lung cancer at initial diagnosis were included. Patients with a recent history of cancer (i.e. malignancy within five years before NSCLC diagnosis, except for skin tumors other than melanoma and non-invasive tumors) were excluded.

Data were matched to PALGA by means of a trusted third party (ZorgTTP, Houten, the Netherlands). PALGA has nationwide coverage since 1991 and contains excerpts of all Dutch pathology reports of histological and cytological examinations [14]. The data request was approved by the scientific and privacy committees of IKNL and PALGA.

2.2. Data extraction and handling

Data on molecular tumor status established in routine practice in pathology laboratories across the Netherlands was extracted manually from relevant pathology reports. These are observational data of incident cases and therefore include both primary and metastatic tumor lesions evaluated by cytology, biopsy or resection specimens, whichever was available. Extracted data included molecular testing for EGFR, KRAS and ALK, date of obtaining the tissue for molecular testing, and mutation/rearrangement status. In routine pathology practice, different techniques were used, including high resolution melting (HRM), (Sanger) sequencing, quantitative PCR, and next-generation sequencing (NGS) to test for EGFR and KRAS mutations, and fluorescence *in situ* hybridization (FISH) and immunohistochemistry (IHC) to test for ALK rearrangement. Tumors with molecular alterations identified on pathology material obtained ≥3 months after diagnosis, with multiple molecular alterations, or with alterations in other driver genes were excluded.

Four molecular subgroups were defined: EGFR+, KRAS+, ALK+, and triple-negative. Triple-negative was defined as negative for all three genes, or EGFR/KRASwt without ALK testing, as only 50% of EGFR/KRASwt tumors underwent ALK testing in 2013 (Kuijpers et al. submitted). Furthermore, as ALK+ is relatively uncommon (~5%), the number of actual ALK+ tumors in this subgroup was expected to be minimal. EGFR mutations were categorized into classic activating mutations (exon 19 deletions and exon 21 L858R point mutations), non-classic activating mutations, resistance mutations, and other mutations (i.e. for which in literature no information on EGFR-TKI sensitivity was available).

2.3. Statistical analysis

Statistical analysis was performed with SPSS (version 20; SPSS Inc., Chicago, IL). Patient characteristics were described according to molecular status, and differences were assessed by *t*-test or X^2-test when applicable. For each organ of metastasis, proportions of tumors metastasized to this organ were, per molecular subgroup (EGFR+, KRAS+, and ALK+), compared to the triple-negative subgroup, and clinicopathological variables age (continuous), gender, histology (adenocarcinoma vs. NSCLC-NOS), and local disease status (≤T2 and ≤N1 vs. ≥T3 and/or ≥N2, excluding cases with unknown T-stage) were taken into account. Only the classic activating EGFR+ were included in the analyses. Crude odds ratios (OR) with 95% confidence intervals (CI) were calculated by univariable logistic regression analyses with triple-negative as the reference category, and variables with a p-value < 0.2 were included in backward multivariable logistic regression analyses to calculate adjusted ORs with 95% CIs. The same was done for the subtypes of classic EGFR mutations and KRAS mutations with an incidence of > 50.

OS was calculated from day of diagnosis till death. Median OS was compared between molecular subgroups and in the EGFR+, KRAS+ and triple-negative subgroups between organs of metastases by standard Kaplan–Meier analysis, which does not control for confounders, and tested for significance with Log-rank test. Patients who were alive at December 31 2015 or who were lost-to-follow-up were censored at last date of follow-up.

3. Results

3.1. Included patients

In 2013, a total of 8608 NSCLC were identified from the NCR, of which 5462 (63.4%) were adenocarcinoma or NSCLC-NOS, and 3323 (60.8%) of those were stage IV (for 37/5462 tumors (0.7%), stage could not be assessed). Eventually, 2052 tumors (2052 patients) were included: 218 EGFR+, 784 KRAS+, 42 ALK+, and 1008 triple-negative (486/1008 with unknown ALK status). Reasons and numbers of excluded tumors are summarized in Fig. 1. All included tumors were pathologically confirmed (either the primary tumor, lymph node or

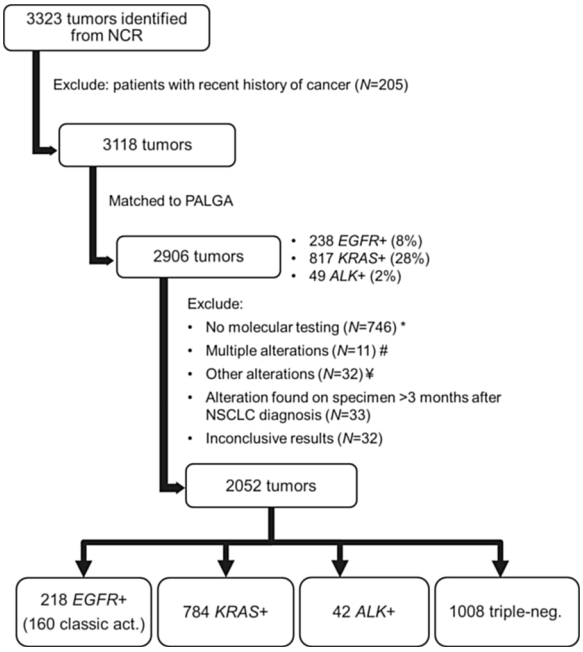

Fig. 1. Flowchart of tumor inclusion.
*No molecular testing or only of one or two genes with negative or indefinite results, except for those tested and negative for EGFR and KRAS.
8 tumors with EGFR+ and KRAS+, 1 with EGFR+ and ALK+, and 2 with KRAS+ and ALK+.
¥ 14 BRAF mutations, 4 PIK3CA mutations, 4 NRAS mutations, 4 ROS-1 rearrangements, 4 HER-2 mutations/translocations, and 2 c-MET amplifications

distant metastasis), and 1784 tumors (89.5%) were histologically confirmed.

Of the EGFR+, 160 (73%) were classic activating mutations (90 exon 19 deletions and 70 exon 21 L858R point mutations), 19 (9%) non-classic activating mutations, 31 (14%) resistance mutations, and 8 (3%) other mutations. Of the KRAS+, 587 (75%) had a mutation in codon 12 (most commonly G12C (n = 303), G12 V (n = 129), G12A (n = 63), and G12D (n = 59)), 53 (7%) in codon 13, 27 (3%) in codon 61, 1 (0.1%) in codon 68, and for 116 patients (15%), the specific KRAS mutation was not reported.Mean age of KRAS+ (P = 0.037) and ALK+ patients (P = 0.006) was significantly lower than of triple-negative patients (Table 1). The EGFR+ and KRAS+ subgroups contained significantly more female patients and adenocarcinomas than the triple-negative subgroup (P < 0.0001). EGFR+ had significantly lower local disease status than triple-negative (P = 0.049).

In 701 cases (35.2%), the mutation/rearrangement was observed (or tested for in case of triple-negative result) in a cytology specimen only and in 1293 cases (64.8%) in a histology specimen (with or without cytology). In 828 cases (41.5%) the mutation/rearrangement was observed (or tested for in case of triple-negative result) in a primary tumor specimen (either alone or in combination with testing in a (lymph node) metastasis), and in 1166 cases (58.5%) in a (lymph node) metastasis specimen only.

3.2. Association of molecular status and metastatic organs

The most common organs of metastases were the bone (N = 1320; 33.8%), pleura (N = 469; 23.5%), lung (N = 460; 23.1%), brain (N = 438; 22.0%), adrenal glands (N = 363; 18.2%), liver (N = 273; 13.7%), and extrathoracic lymph nodes (N = 226; 11.3%).

Compared to triple-negative patients, EGFR+ patients had significantly more often bone metastases (53.8% vs. 31.5%); OR 2.55 (95% CI 1.80–3.62), pleural metastases (37.5% vs. 24.1%; OR 2.06 (1.42–2.98)), and less often brain metastases (12.5% vs. 22.0%; OR 0.53 (0.32–0.88)) and adrenal gland metastases (7.5% vs. 19.1%; OR 0.37 (0.20–0.68)) (Fig. 2). KRAS+ patients had significantly more often lung metastases (26.7% vs. 20.3%; OR 1.40 (1.12–1.76)) than triple-negative, and ALK+ patients showed a trend towards more often liver metastases (23.8% vs. 12.9%; OR 2.07 (1.00–4.32)) than triple-negative patients.

The most common combinations of organs with metastases were bone + adrenal glands (N = 129; 6.5%), bone + liver (N = 115; 7.8%), bone + lung (N = 115; 7.8%), bone + pleura (N = 93; 4.7%), and brain + adrenal glands (N = 65; 3.3%). Compared to triple-negative patients, EGFR+ patients had significantly more often a combination of bone with liver (11.3% vs. 4.7%); OR 2.59 (95% CI 1.46–4.59), lung (10.6% vs 4.8%); OR 2.21 (95% CI 1.23–3.96), and pleural metastases (9.4% vs. 4.5%); OR 2.46 (95% CI 1.32–4.57). ALK+ patients also had significantly more often a combination of bone and liver metastases than triple-negative patients (11.9% vs. 4.7%; OR 2.76 (1.04–7.35)),

Table 1
Characteristics of the included tumors.

	EGFR+ [#] (n = 160)	*KRAS*+ (n = 784)	*ALK*+ (n = 42)	Triple-negative (n = 1008)
Age; mean (SD)	66.7 (11.7)	64.1 (9.7)*	60.4 (12.2)**	65.1 (10.9)
Gender				
Male	36.9%***	44.9%***	57.1%	61.4%
Female	63.1%	55.1%	42.9%	38.6%
Histology				
Adenocarcinoma	98.1%***	92.0%***	95.2%	85.5%
NSCLC-NOS	1.9%	8.0%	4.8%	14.5%
N-stage				
N0-1	28.7%	24.1%	21.4%	23.8%
N2-3	71.3%	75.9%	78.6%	76.2%
Local disease status[¥]				
≤T2 and ≤N1	16.9%*	11.0%	7.1%	11.3%
≥T3 or ≥N2	80.6%	86.2%	90.5%	85.4%
T unknown	2.5%	2.8%	2.4%	3.3%
Number of organs with metastases				
1	46.9%	50.8%	45.2%	54.3%
2	33.1%	27.6%	40.5%	27.8%
≥3	20.0%	21.7%	14.3%	18.0%
PET imaging performed				
Yes	9.4%	8.8%	4.8%	9.2%
Unknown	90.6%	91.2%	95.2%	90.8%

[#] Classic activating *EGFR* mutations.
[¥] Unknown T-stage excluded from comparison.
* *P*-value compared to triple negative < 0.05.
** < 0.01.
*** < 0.001.

3.3. Association of molecular driver subtypes and metastatic organs

Compared to triple-negative patients, those with an EGFR exon 19 deletion and L858R mutation more often had bone metastases (exon 19 deletion: 47.8%; OR 2.05 (1.31–3.20); L858R: 61.4%; OR 3.40 (2.04–5.66)) and pleural metastases (exon 19 deletion: 36.7%; OR 2.02 (1.26–3.26); L858R: 38.6%; OR 2.10 (1.25–3.54)) (Fig. 3). Furthermore, patients with an EGFR exon 19 deletion less often had adrenal gland metastases (5.6% vs. 19.1%; OR 0.27 (0.11–0.67)) and patients with an L858R mutation less often had brain metastases (10.0% vs. 22.0%; OR 0.44 (0.20–0.98)).

Patients with a KRAS G12A mutation more often had metastatic disease to the bone than triple-negative patients (42.9% vs. 31.5%; OR 2.26 (1.33–3.81)) and with a G12 V mutation more often to the lung (29.5% vs. 20.3%; OR 1.60 (1.05–2.45)) (Fig. 4).

3.4. Overall survival in relation to molecular status and metastatic organs

Median follow-up of censored cases was 30.2 months. Median OS

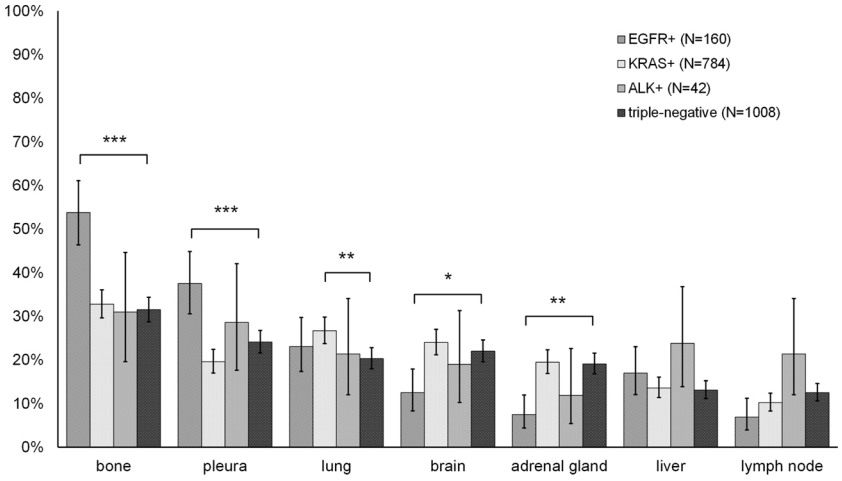

Fig. 2. Comparison between molecular subgroups in the proportions of tumors metastasized to the seven most common metastatic organs.
*P-value compared to triple negative < 0.05, ** < 0.01, *** < 0.001 adjusted for clinicopathological variables (bone: gender, histology and local disease status; pleura: gender, age, histology and local disease status; lung: age, histology and local disease status; brain: gender, age, histology and local disease status; adrenal gland: age, histology and local disease status; liver: none; lymph node: age and local disease status).

was significantly higher for EGFR+ (18.2 months) and ALK+ (15.4 months), but not for patients with KRAS+ NSCLC (8.8 months), as compared to triple negative disease (8.9 months) ($p < 0.001$). In all molecular subgroups, liver metastasis was associated with worse OS compared to absence of liver metastases (Table 2). Only in KRAS+ NSCLC, bone, pleural, and adrenal gland metastases were associated with worse OS as well.

4. Discussion

In this nationwide population-based study of treatment-naive stage IV ns-NSCLC we evaluated the association of molecular status and metastatic organs. Classic activating EGFR+ was associated with a higher frequency of bone and pleural metastases and a lower frequency of brain and adrenal gland metastases as compared to triple negative tumors. KRAS+ had a higher frequency of lung metastases, whereas ALK+ showed a higher frequency of liver metastases than triple-negative tumors.

The lower frequency of brain metastases in EGFR+ patients is in contrast to previous studies [5,7–9]. In the Dutch guideline on NSCLC, brain imaging is only advised in symptomatic patients [13]. Data on neurological symptoms or brain imaging were not available to us. Hence, some preclinical brain metastases might be missed, but we assume the distribution of patients without brain imaging to be similar between the molecular subgroups.

We confirmed the higher frequency of bone metastases in EGFR+ patients reported by others [6,8]. As 54% of the EGFR+ stage IV ns-NSCLC patients had bone metastases at diagnosis, a prospective trial screening all EGFR+ patients for bone metastases is worth considering. This advice would also fit in the current ESMO clinical practice guideline [15], which states that only selected NSCLC patients, with symptomatic or asymptomatic bone metastases, with a life expectancy > 3 months, and considered at high risk of skeletal related events (SREs), should be treated with zoledronic acid or denosumab to prevent SREs. However, it is not defined in this guideline which specific type of NSCLC patient fits this description, and our results may help in defining potential candidates. Results regarding the risk of SREs in EGFR+ patients are conflicting and it is currently not clear whether EGFR-TKI can prevent all SREs [11,16]. In vitro, EGFR signaling plays an important role in osteoclastogenesis and RANKL activation, and RANKL transactivates EGFR [17]. Adding a bone targeted agent to EGFR-TKI therapy might decrease the risk of SREs in EGFR+ patients. Bisphosphonates were shown to enhance the EGFR-TKI antitumor effect [18]. The RANKL inhibitor Denosumab might also act synergistically with EGFR-TKI, but, to our knowledge, no (preclinical) data exist.

Also in line with previous studies are the lower incidence of adrenal gland metastases [6] and the higher incidence of pleural metastases in EGFR+ patients vs. triple-negative patients [19,20], and the higher incidence of lung and liver metastases in KRAS+ [21] and ALK+ [6,10] patients, respectively, vs. triple-negative patients. KRAS+ G12A tumors had more often metastases in the bone than triple-negative tumors. In literature, the risk of bone being the first site of recurrence or metastasis was higher in G12C mutated surgically resected tumors than in wt, EGFR+, and other KRAS+ tumors, but only 2 tumors with a G12A mutation were described [7].

Strengths of this study are the inclusion of only treatment-naive

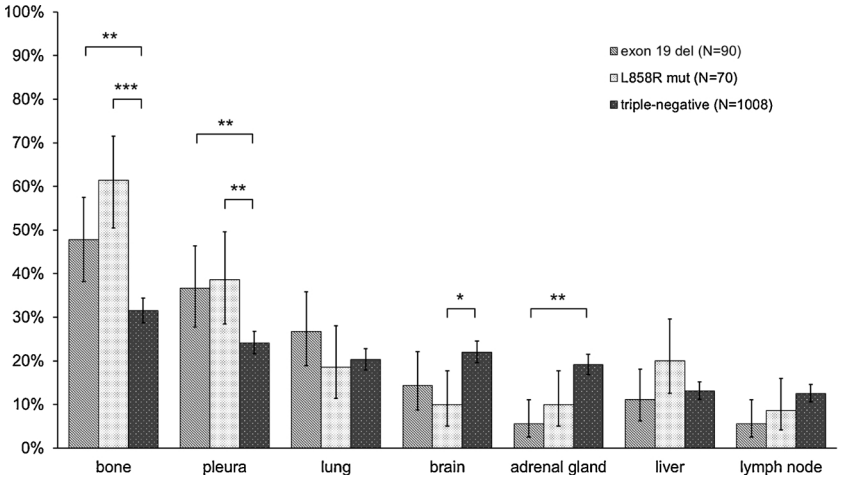

Fig. 3. Comparison between subtypes of EGFR classic activating mutations in the proportions of tumors metastasized to the seven most common metastatic organs.
*P-value compared to triple negative < 0.05, ** < 0.01, *** < 0.001 adjusted for clinicopathological variables (bone: gender, histology and local disease status; pleura: gender, age, histology and local disease status; lung: age, histology and local disease status; brain: gender, age, histology and local disease status; adrenal gland: age, histology and local disease status; liver: none; lymph node: age and local disease status).

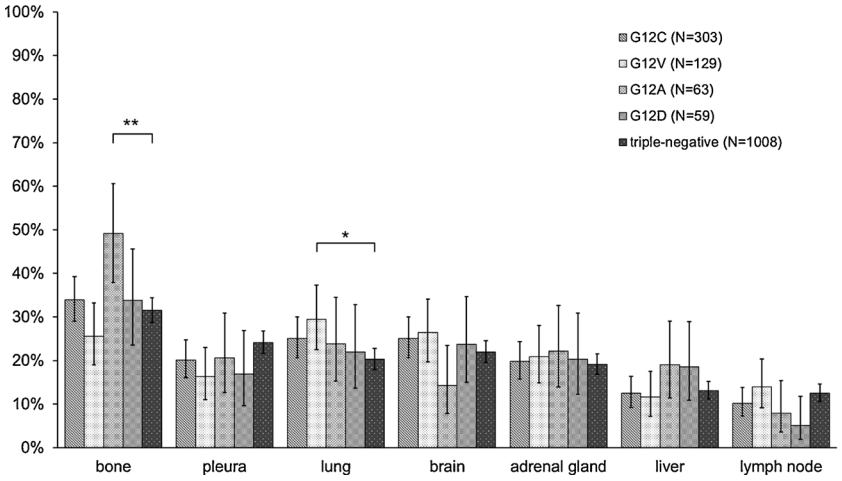

Fig. 4. Comparison between subtypes of KRAS mutations in the proportions of tumors metastasized to the seven most common metastatic organs.
*P-value compared to triple negative < 0.05, ** < 0.01, *** < 0.001 adjusted for clinicopathological variables (bone: gender, histology and local disease status; pleura: gender, age, histology and local disease status; lung: age, histology and local disease status; brain: gender, age, histology and local disease status; adrenal gland: age, histology and local disease status; liver: none; lymph node: age and local disease status).

Table 2
Median overall survival (OS) in months per molecular subgroup compared between patients with and without metastatic disease in the organ of interest (by Kaplan-Meier analysis).

	EGFR +			KRAS +			triple-negative		
	meta +	meta-	*P*-value	meta +	meta-	*P*-value	meta +	meta-	*P*-value
bone	18.5	17.8	ns	6.8	9.8	< 0.001	7.8	9.0	ns
pleura	16.4	19.2	ns	6.8	9.3	< 0.001	8.1	8.8	ns
lung	20.1	17.7	ns	11.1	7.9	< 0.001	8.3	8.7	ns
brain	15.5	18.3	ns	9.1	8.7	ns	9.6	8.4	ns
adrenal gland	14.8	18.5	ns	5.7	9.5	< 0.001	7.8	8.8	ns
liver	10.0	19.6	< 0.001	5.8	9.3	< 0.001	5.8	9.0	< 0.001
lymph node	15.9	18.2	ns	8.3	8.8	ns	8.4	8.6	ns

stage IV ns-NSCLC patients, excluding bias from previous treatments, and its population-based character using uniformly registered data from nationwide registries, excluding selection bias (which might be the case in single-centerstudies) and enabling inclusion of large numbers of patients.

This study has a few limitations. First, the number of ALK + patients was still relatively low, possibly because in the Netherlands in 2013 only 24% of stage IV ns-NSCLC were tested for ALK rearrangement, representing 50% of the EGFR/KRASwt tumors (Kuijpers et al., submitted). Patient numbers per specific molecular driver subtype (e.g. KRAS G12A, EGFR exon 19 deletion) were small and no definitive conclusions can be drawn for these specific subtypes. Second, EGFR/KRASwt tumors without ALK testing were included as triple-negative, but similar results were obtained when these tumors were excluded from analyses. Third, only classic EGFR + were included, but analysis of all EGFR + produced similar results (data not shown). Bias due to the type and site of available specimens is not expected. The proportions of tumor samples with a tumor cell percentage of ≤10%, ≤20% and ≤30%, and the proportion of tumors where the mutation/rearrangement was observed in cytology only (or tested for in case of triple-negative) did not differ significantly between tumors with and without a driver mutation (i.e. EGFR + /KRAS + /ALK + vs. triple-negative). In addition, although triple-negative tumors had significantly more often molecular testing performed on (lymph node) metastasis specimens only than tumors with a driver mutation/rearrangement (61.5% vs 55.4%; P = 0.005), triple-negative patients were deemed truly triple-negative, as mutations are in principle truncal, and therefore expected to be present in the primary tumor and in the metastases. Fourth, only up to three organs with metastatic sites are coded in the NCR. When there are metastases in ≥3 organs, two are recorded and the third is coded as ≥3, This might result in bias regarding reporting of organs with specific metastases. However, as all data managers are trained to score in the same format,we presume that this did not result in reporting bias between the different molecular groups. Finally, data on ^{18}FDG-PET scan performance, which can detect more metastases than CT alone, was only available for a limited number of patients (evenly distributed between the molecular subgroups). Reporting of staging procedures such as ^{18}FDG-PET, bone scan or magnetic resonance imaging (MRI) is an optional instead of a mandatory item in the NCR. It is therefore probable that a number of ^{18}FDG-PET staged patients were not scored as such in our analysis. Reporting of metastatic organs depended on available imaging information. The lack of detailed staging information is a limitation of our study, however, staging procedures are not dependent on molecular status and according to the Dutch NSCLC guideline all patients should receive at least a CT of the chest and upper abdomen, and therefore we assume that this will not have caused bias with regard to reporting organs of metastases. Moreover, the NCR does not include data on WHO performance status (PS) of the patients while this is of prognostic significance. However, in the proposals for the M-descriptors of the 8th TNM classification [22], ^{18}FDG-PET and WHO PS data were also not available, but still M1a/M1b showed better outcomes than M1c, suggesting that results without ^{18}FDG-PET staging data and WHO PS data can be used for these kind of analyses.

5. Conclusion

In conclusion, molecular status of NSCLC is associated with organs of metastasis at diagnosis. 54% of stage IV EGFR + ns-NSCLC patients had bone metastases at diagnosis. These observational results are hypothesis generating, and call for a prospective study in which EGFR + patients are screened for bone metastases, and treated to prevent SREs.

Conflict of interest statement

CK and SW received funding from Roche and Pfizer, but Roche and

Pfizer had no role in study design, analyses and reporting. AD attended advisory boards from Roche, Lilly, Clovis, AstraZeneca, MSD, Boehringer Ingelheim, fees were paid to her institute. All remaining authors have declared no conflicts of interest.

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